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Calcium Regulating Hormones after Oral and Intravenous Calcium Administration

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Summary: The aim of this study was to determine the changes in serum calcium concentration and in the concentrations of calcium regulating hormones after a single oral or intravenous calcium administration. Standard dosages of calcium, as used in routine patient care, were employed.

Intact parathyrin, calcitonin, calcitriol, calcidiol, total calcium, ionized calcium, total protein and phosphate were determined in 12 healthy young men before and up to 8 h after oral and intravenous administration of calcium.

During a fortnight there were four study days with 1000 mg calcium orally (p. o.), 2000 mg orally, 180 mg calcium intravenous (i. v.) and a control day without calcium. During the study the men were on a low calcium diet.

We observed a sharp increase in the calcium concentration after i. v. administration (15 min: total Ca: $+0.48 \pm 0.32$ mmol/l; ionized Ca: $+0.25 \pm 0.15$ mmol/l; $p < 0.01$). The concentration increase after the two oral loads was nearly identical. The maximal concentration of total calcium was reached after 120 min (1000 mg: $+0.1 \pm 0.04$ mmol/l; $p < 0.001$; 2000 mg: $+0.12 \pm 0.04$ mmol/l; $p < 0.001$). There was a significant increase in urinary calcium after all modes of calcium administration.

Calcitonin increased significantly only after i. v. injection of calcium ($+9.2 \pm 3.4$ pmol/l; $p < 0.001$) while parathyrin decreased significantly after all modes of calcium administration (i. v.: 15 min: -1.9 ± 0.88 pmol/l; $p < 0.01$; 1000 mg: 90 min: -0.78 ± 0.75 pmol/l; $p < 0.001$; 2000 mg: 90 min: -1.02 ± 0.57 pmol/l; $p < 0.001$). Almost total suppression of parathyrin secretion occurred at the upper end of the physiological range for serum calcium. There was no statistically relevant change of either calcidiol or calcitriol throughout each study day.

The phosphate concentration showed an earlier increase to a higher afternoon level after all three modes of calcium administration, compared with the control day.

In conclusion, both the oral or intravenous administration of calcium significantly influence the regulation of calcium metabolism. Both oral dosages yielded an increase of serum calcium with depression of parathyrin secretion without major risk of hypercalcaemia. Suppression of intact parathyrin for evaluation of patients with suspected hyperparathyroidism may easily be effected by oral ingestion of 1000 mg calcium.

Introduction

Calcium absorption after oral calcium loading has been investigated by various authors (1–7). The results of these studies were controversial with regard to the fraction of intestinal calcium absorption in relation to the calcium load. In some of the studies, a change in fractional absorption after different amounts of calcium was observed (1, 6, 7), whereas this was not found by *Behne* et al. (3).

Numerous studies have also been reported on the effects of calcium on parathyroid hormone in vitro and in vivo (8–22) or its interference with other calcium regulating hormones (2, 23). Many of these studies showed the sigmoidal relationship between calcium and parathyrin (9–12, 16, 18).

Intravenous administration of calcium resulted in a strong suppression of the parathyrin secretion rate to 40% of the mean basal concentration (14, 20). Oral calcium administration caused a reduction of parathyrin release by 50% (15, 21) or up to 73% (22). Parathyrin was often measured only with radioimmunoassays for parathyroid hormone fragments (14, 17, 22), which are not as sensitive as the sandwich-type assays for the intact hormone (15, 20, 21). Because of the short half-life of the intact hormone (24, 25) its measurement reflects the physiological state of the parathyroid gland more accurately than the determination of parathyroid fragments, whose concentration is considerably influenced by kidney function. To our knowledge, the effect of oral and intravenous administration of calcium in different doses on intact parathyroid hormone and other calcium regulating hormones has not been determined in detail.

Using a sensitive assay for intact parathyrin and measuring all calcium regulating hormones, our aim was to determine the way in which different modes of administration and different dosages affect serum calcium concentrations and calcium regulating hormones, especially the secretion of intact parathyrin. We administered standard calcium preparations of the type often used in routine patient care.

Materials and Methods

Study subjects

Twelve healthy men (mean age 26.4 a; range 21–45 a) volunteered for this study, which was approved by the ethics committee of the University of Heidelberg. All subjects were in good physical condition, and no abnormalities in serum calcium, phosphate, alkaline phosphatase, total protein, urea and creatinine were observed. No subject took any other drugs during and at least one week before the study.

Materials

We used Calcium-Sandoz® fortissimum tablets for oral administration. One tablet contains 1000 mg calcium. The tablets were dissolved in water and ingested. The volunteers took one tablet for the 1000 mg and two tablets for the 2000 mg dose.

For intravenous administration we used Calcium-Sandoz® injection solution 20% containing 180 mg calcium in a 10 ml ampoule.

Study course

The study was performed within 14 days in each volunteer. It consisted of 4 blood sampling periods with intervals of 2 or 3 days when the volunteers were on a low calcium diet (no milk products) without suffering from calcium hunger (no elevation of intact parathyrin was observed). On the four sampling days they had a low-calcium breakfast at 0800 h (bread, 15 g of butter, marmalade: total amount of calcium < 50 mg) and a low-calcium lunch after the 1300 h collection (rice and vegetables: total amount of calcium < 100 mg). Control samples were obtained from each volunteer before the administration of calcium. On the following study days, three different calcium doses (1000 mg p. o., 2000 mg p. o., or 180 mg i. v.) were applied in a randomized order.

An indwelling catheter was placed in a forearm vein at 0845 h and a blood sample was taken. All subjects received their calcium dose at 0900 h. The calcium tablets were dissolved in water and ingested. The 180 mg calcium gluconate dose was slowly injected within 5 min through the indwelling catheter. Blood samples were taken every 15 min in the first hour, every 30 min in the following two hours, and hourly in the remaining five hours. Each time we sampled about 7 ml EDTA-plasma and closed the catheter with a stylet. Additionally, 10 ml blood were taken before the first sampling after the i. v. administration to clean the indwelling catheter.

The blood samples were centrifuged within 4 h and stored frozen at −30 °C until assayed. For the ionized calcium series, an additional 2 ml of blood were taken in a syringe, and analysed immediately.

Urine was sampled at 0900 h (spot sample), 1300 h and 1700 h (both four hour samples). Before measuring, HCl was added and the urines were heated to dissolve calcium salts.

Clinical chemical analyses

Serum calcium and urinary calcium were determined by atomic emission spectrophotometry corrected for sodium interference (CAFM, Eppendorf Gerätebau, Hamburg, Germany). The protein-adjusted calcium concentration was calculated by a formula of *Husdan* (26).

The ionized calcium and pH values were determined with an ion selective electrode (Ionized Calcium Analyser ICA 2, Radiometer Copenhagen) immediately after the blood sample had been drawn. The measured ionized calcium value was automatically adjusted to a pH of 7.4.

Serum phosphate, alkaline phosphatase, total protein, urea, creatinine, sodium, potassium, and urine creatinine were determined with a Hitachi-737 analyser. Reagents were obtained from the Boehringer Mannheim Corporation, Mannheim, Germany.

Calcitonin

Calcitonin was determined with an immunoradiometric assay (IRMA) similar to the method of *Motté* et al. (27) (*M. Engelbach*, in preparation).

Parathyrin

Intact parathyrin concentrations were determined in duplicate using a two-site immunoradiometric assay (IRMA). This assay will be described in detail by *Herfarth* et al. (in preparation). It uses a polyclonal anti-human parathyrin (1–34) antibody raised in a rabbit and a monoclonal anti-human parathyrin (44–68) antibody (Mab 14H5) from Medgenix, Brussels, Belgium (28). This IRMA recognizes only the intact parathyrin molecule and has no cross-reactivity with hormone fragments in the physiological range. The sensitivity of this assay was 0.13 pmol/l. The intra-assay variation coefficients were below 7.3% in the normal range. The normal concentrations ranged from 0.8 to 5.3 pmol/l (mean 2.77 ± 1.18 pmol/l; median 2.7 pmol/l).

Calcitriol

Calcitriol was determined with a radioimmunoassay after high performance liquid chromatography of serum extracts according to *Scharla* et al. (29).

Calcidiol

Calcidiol (25-hydroxycholecalciferol) was determined with a competitive protein-binding assay without chromatography according to *Bothe* et al. (30).

Statistics

For statistical analysis *Student*'s t-test for paired samples was employed.

Results

Total calcium

A sharp increase in the serum calcium concentration (protein-adjusted) of 0.48 ± 0.32 mmol/l ($p < 0.05$; range: 0.28–1.47 mmol/l) was observed 15 min after i.v. administration of 180 mg calcium as calcium gluconate. Calcium decreased quickly but remained at an elevated level for the 480 min of observation (min 480: +0.03 ± 0.04 mmol/l (mean ± SD); $p < 0.01$).

A smaller increase in serum calcium concentration (protein adjusted) was observed after oral administration. After a 1000 mg calcium load, a statistically significant increase was noted at 30 min (+0.05 ± 0.03 mmol/l; $p < 0.05$) and the maximal concentration of serum calcium (protein-adjusted) occurred after 120 min (+0.1 ± 0.04 mmol/l; $p < 0.001$; range: 0.03–0.15 mmol/l). The 2000 mg calcium load also produced a statistically significant increase at 30 min (+0.07 ± 0.05 mmol/l; $p < 0.05$) and a maximal increase after 120 min (+0.12 ± 0.04 mmol/l; $p < 0.001$; range: 0.07–0.17 mmol/l). Both doses resulted in increased serum concentrations until the end of observation (min 480: 1000 mg: +0.03 ± 0.05 mmol/l; $p < 0.001$; 2000 mg: +0.04 ± 0.04 mmol/l; $p < 0.001$). There were no differences between the calcium concentrations of persons receiving 1000 mg at the beginning and those receiving the same dosage at the end of the test period. Similarly, the changes in calcium concentrations after the other modes of application did not differ between the beginning and the end of the test period. The concentrations of total calcium during the four study days are shown in figure 1.

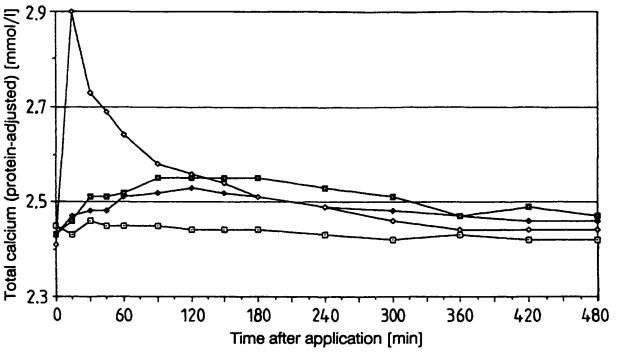

Fig. 1. Total calcium concentrations (protein-adjusted) on the control day and after the three different modes of calcium application. (As the figures seemed overloaded by the introduction of error bars, these were omitted.)
Calcium application:
—⊡— control
—◆— 1000 mg oral
—□— 2000 mg oral
—◇— 180 mg i.v.

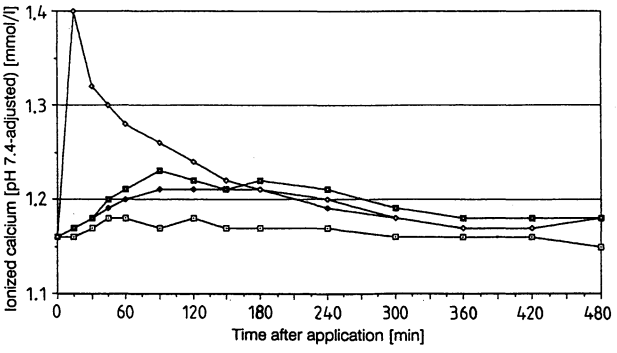

Fig. 2. Ionized calcium concentrations (pH 7.4 adjusted) on the control day and after the three different modes of calcium application.
Calcium application:
—⊡— control
—◆— 1000 mg oral
—□— 2000 mg oral
—◇— 180 mg i.v.

Ionized calcium (pH-adjusted)

The ionized calcium determinations (pH adjusted) yielded similar results (fig. 2). A sharp increase occurred after i.v. administration with a maximal concentration after 15 min (+0.25 ± 0.15 mmol/l; $p < 0.01$; range 0.15–0.69 mmol/l), and a slight in-

crease of ionized calcium was found after oral administration with a statistically significant increase at 60 min (1000 mg: +0.04 ± 0.02 mmol/l; $p < 0.05$; 2000 mg: +0.05 ± 0.04 mmol/l; $p < 0.1$) and a maximal concentration after 120 min (1000 mg: +0.06 ± 0.02 mmol/l; $p < 0.01$; range: 0.02–0.12 mmol/l) and 90 min (2000 mg: +0.07 ± 0.04 mmol/l; $p < 0.05$; range: 0.01–0.14 mmol/l). The ionized calcium concentrations remained elevated after all three modes of administration until the end of the observation period (min 480: all: +0.02 mmol/l; $p < 0.05$).

Urinary excretion

There was an increase of urinary calcium excretion after all three modes of administration (1000 mg p.o.: $p < 0.05$; 2000 mg p.o.: $p < 0.05$; 180 mg i.v.: $p < 0.001$) (fig. 3). A mean calcium concentration of 9.82 ± 5.11 mmol/g creatinine (1.11 ± 0.58 mmol/mmol creatinine) was observed after the intravenous dosage. This was higher than the excretion after the 1000 mg (7.57 ± 3.57 mmol/g creatinine (0.86 ± 0.4 mmol/mmol creatinine)) or the 2000 mg (6.62 ± 1.31 mmol/g creatinine (0.75 ± 0.15 mmol/mmol creatinine)) oral dose, but the difference was not statistically significant. In the late afternoon the urinary calcium excretion rates after all three modes of administration were statistically still significantly higher than on the control day.

Further data on urinary calcium excretion are shown in table 1.

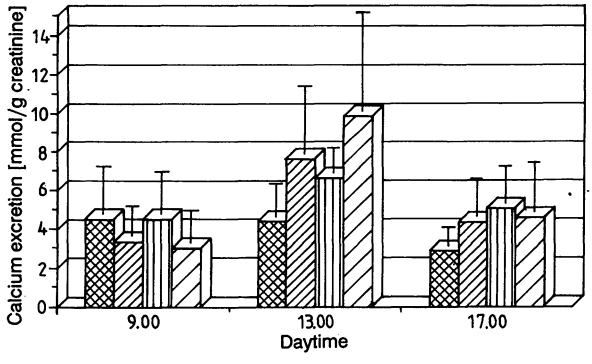

Fig. 3. Urinary calcium excretion on the control day and after the three different modes of calcium application at the different sampling times. The error bars represent the standard error. (Data of 2 indidviduals were not available after the i.v. and the 1000 mg dosage and of 3 individuals after the 2000 mg dosage.)
Calcium application:
▩ control
▨ 1000 mg oral
▥ 2000 mg oral
▨ 180 mg i.v.

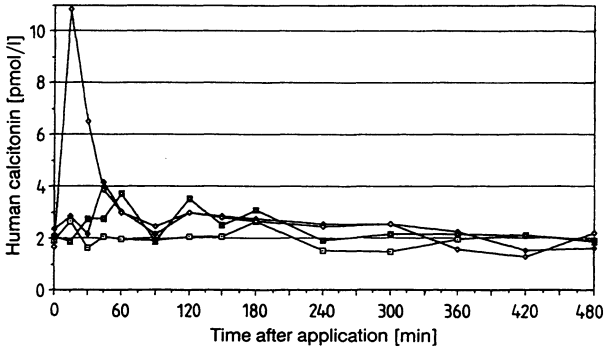

Fig. 4. Calcitonin concentrations on the control day and after the three different modes of calcium application.
Calcium application:
—□— control
—◆— 1000 mg oral
—□— 2000 mg oral
—◇— 180 mg i.v.

Calcitonin

Calcitonin concentrations increased significantly only after the i.v. administration of calcium (fig. 4). The maximal concentration was found after 15 min with a difference of 9.2 ± 3.37 pmol/l versus the concentration before administration ($p < 0.001$; range: 4.2–13.5 pmol/l). It remained significantly elevated for 45 min ($p < 0.01$). Significant concentration changes of calcitonin were not observed after oral administration of either 1000 mg or 2000 mg calcium.

Parathyrin

A significant decrease of parathyrin secretion occurred immediately after i.v. administration (fig. 5).

Tab. 1. Mean values of urinary calcium concentrations and mean four hour calcium excretion (normal calcium excretion: < 7.5 mmol/24 h) on the control day and after the three different modes of calcium application.

	Calcium-excretion [mmol/l]				Calcium-excretion [mmol/4 h]			
	Control	1000 mg oral	2000 mg oral	180 mg i.v.	Control	1000 mg oral	2000 mg oral	180 mg i.v.
0900 h	4.44 ± 2.65	4.86 ± 3.76	4.93 ± 2.85	4.53 ± 1.95	–	–	–	–
1300 h	4.68 ± 2.18	6.65 ± 3.34	6.87 ± 2.46	8.14 ± 3.2	1.19 ± 0.49	2.18 ± 1.04	2.28 ± 0.6	2.73 ± 1.15
1700 h	3.76 ± 1.2	5.23 ± 1.73	5.26 ± 1.79	5.81 ± 3.4	0.84 ± 0.26	1.5 ± 0.72	1.94 ± 0.69	1.53 ± 0.95

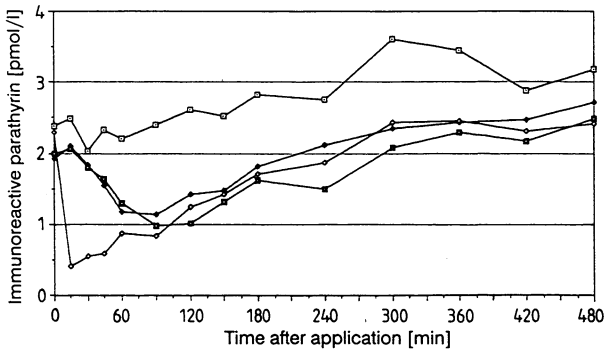

Fig. 5. Parathyroid hormone concentrations on the control day and after the three different modes of calcium application.
Calcium application:
—⊡— control
—◆— 1000 mg oral
—□— 2000 mg oral
—◇— 180 mg i. v.

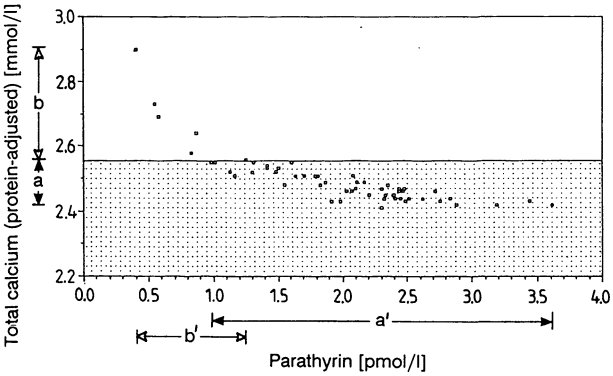

Fig. 6. Mean total calcium concentrations (protein-adjusted) (y-axis) versus mean parathyroid hormone concentrations (x-axis). The shadowed area indicates the physiological calcium range. One dot refers to the mean data of one sampling time. In the range of 2.41 to 2.55 mmol/l of serum calcium the parathyroid glands responded to produce a range of intact parthyrin concentrations, in which the highest and lowest values differed by 2.63 pmol/l (see a and a'). Above the physiological calcium range a calcium increase of 0.34 mmol/l is followed by a parathyrin decrease of only 0.85 pmol/l (see b and b').

The lowest concentrations were found after 15 min with a mean decrease of 1.9 ± 0.88 pmol/l ($p < 0.01$; range: (−1.0)−(−3.8) pmol/l). Parathyrin concentrations remained low for 240 min ($p < 0.05$).

A statistically significant response of the glands to the oral administration was observed, the lowest parathyrin concentrations occurred at 90 minutes. The mean parathyrin concentration decreased by 0.78 ± 0.75 pmol/l ($p < 0.001$; range: (−2.2)−(+0.3) pmol/l) after 1000 mg and by 1.02 ± 0.57 pmol/l ($p < 0.001$; range: (−2.0)−(−0.1) pmol/l) after 2000 mg of calcium. The parathyrin concentrations remained significantly lowered for 180 min (1000 mg; $p < 0.05$) or 240 min (2000 mg; $p < 0.01$).

Figure 6 shows the mean total calcium concentrations (protein-adjusted) versus the mean parathyrin values. One dot refers to the mean data of one sampling time. In the range of 2.41 to 2.55 mmol/l of serum calcium the parathyroid glands responded to produce a range of intact parathyrin concentrations, in which the highest and lowest values differed by 2.63 pmol/l (see a and a'). All the calcium concentrations above the normal range were the result of intravenous administration. The parathyroid glands cannot respond to these high calcium concentrations in the same manner as they do in the physiological range: a calcium increase of 0.34 mmol/l is followed by a parathyrin decrease of only 0.85 pmol/l (see b and b').

Phosphate

The serum phosphate concentrations after calcium administration (i. v. and oral) showed no significant differences in comparison with the control day during the first hours of blood sampling; thereafter, however, the phosphate concentrations rose earlier (fig. 7). The i. v. administration group showed slightly elevated phosphate concentrations after 90 min (+0.01 ± 0.22 mmol/l; $p < 0.1$; control: −0.1 mmol/l). A significant difference was observed between 120 min and 180 min (+0.11 ± 0.25 mmol/l; $p < 0.01$; range: (−0.52)−(+0.42) mmol/l). After oral administration of 2000 mg calcium, significant changes were observed between 120 min ($p < 0.05$) and 180 min ($p < 0.01$); after 1000 mg calcium, significant changes were observed between 150 min ($p < 0.05$) and 180 min ($p < 0.01$).

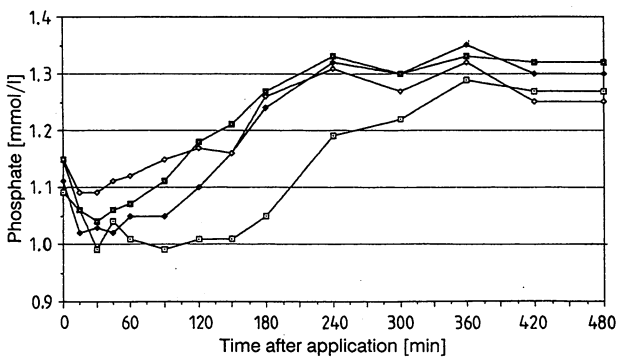

Fig. 7. Phosphate concentration on the control day and after the three different modes of calcium application.
Calcium application:
—⊡— control
—◆— 1000 mg oral
—□— 2000 mg oral
—◇— 180 mg i. v.

Tab. 2. Morning and evening concentrations of calcidiol (25-hydroxycholecalciferol) and calcitriol (1,25-dihydroxycholecalciferol) on the control day and after the three different modes of calcium application.

Calcidiol				
	Control	1000 mg oral	2000 mg oral	180 mg i. v.
Morning mean ± SD [nmol/l]	61.58 ± 27.24	58.67 ± 22.09	61.00 ± 21.27	60.83 ± 26.44
Evening mean ± SD [nmol/l)	63.67 ± 27.93	61.00 ± 24.31	59.58 ± 26.03	62.00 ± 25.11
Calcitriol				
	Control	1000 mg oral	2000 mg oral	180 mg i. v.
Morning mean ± SD [pmol/l]	109.9 ± 16.6	95.8 ± 27.1	106.6 ± 18.0	100.3 ± 20.6
Evening mean ± SD [pmol/l]	105.1 ± 19.4	104.1 ± 20.4	102.5 ± 22.8	90.5 ± 17.3

Calcidiol, calcitriol

No significant changes in the calcidiol and calcitriol concentrations were observed between the beginning and the end of the different study days. The results are shown in table 2.

Total protein, sodium and potassium

Total protein, sodium and potassium showed no significant changes during the sampling time, either on the control day or on the study days.

Discussion

Serum calcium concentrations showed a pronounced initial increase with the risk of hypercalcaemic side-effects if calcium is given intravenously under the described conditions, while both oral dosages lead to a slow but significant long lasting increase of serum calcium concentration within the physiological range.

Urinary calcium increased substantially after calcium administration, so an increase in fluid intake should be recommended for patients under long-term calcium treatment. The excretion rates after both oral dosages were, however, lower than after intravenous injection. For intravenous injection, a 90 mg ampoule is also available. We selected the 180 mg ampoule, because this is nearer to the suggested amount intestinally absorbed from the tablets.

Despite the higher absorption in the non-fasting state (4, 5), the gradual serum calcium increase caused by oral administration was counter-regulated by a slight, though not significant, increase in the calcitonin level and a pronounced decrease in the parathyrin concentration. Due to this sensitive regulation, calcium concentrations almost always remained within the physiological range ($\leq$ 2.55 mmol/l); only one subject had slightly elevated concentrations in two samples. Oral administration of calcium thus appears to be safe in patients without disturbances of calcium metabolism, i. e. there is no risk of hypercalcaemic side-effects.

There were various effects on the calcium regulating hormones. The morning levels of some variables (e. g. parathyroid hormone) differed slightly between the control day and the other study days. After the 2000 mg oral dose of calcium, for instance, the parathyrin concentration showed a reduction to 49.2% of the morning level of the same day but to 38.5% of the morning level on the control day. This differences is due to the higher morning level of parathyrin on the control day and to the increase of parathyrin during the day. This increase of intact parathyrin in the control group during the day was statistically significant ($p < 0.001$) (Herfarth et al., in preparation). Despite this, our data of the parathyrin response to oral calcium confirm previous studies on intact parathyrin (15, 21). The concentration changes of either calcium or parathyrin after the oral load were twice as rapid in our study as that described by *Horowitz* et al. in osteoporotic postmenopausal women (17). They observed the peak of serum calcium concentration after a time of three hours in malabsorbers as well as in normal absorbers. It is suggested that this difference might result from age and sex. It might also reflect a disturbed regulation of calcium and bone metabolism in the special situation of osteoporotic

women. *Epstein* et al. have described a lower absorption of calcium in the elderly caused by vitamin D deficiency, but not a delay in absorption (2). Further studies should be done to establish the clinical relevance of these phenomena.

A significant change in calcitonin concentration was only observed after i. v. administration but not after the oral calcium loads. This might have been due to the assay sensitivity, but it might also be possible that the thyroidal C-cells only cause a significant change in calcitonin secretion at calcium concentrations above the normal range, and that physiological regulation is effected within very narrow calcitonin concentration limits.

As shown in figure 7, an increase of phosphate occurred within one hour after oral or i. v. administration, but this was statistically significant only after two or more hours. For the study of the phosphate concentrations, comparison with the control period was mandatory as there is a physiological decrease of plasma phosphate from 9 a. m. to noon, followed by an increase. The changes in the phosphate concentrations of the control subjects were similar to the observations of *Jubiz* et al. (31) who also found that the physiological increase of phosphate is abolished by fasting.

If the differences from the starting concentration of total and ionized serum calcium are plotted, slight decreases are observed on the control day. We suggest that the increase of phosphate during the afternoon is the primary event and that the decrease of calcium and the increase of parathyrin are secondary events, since a primary parathyrin increase should decrease plasma phosphate. Oral or i. v. calcium seems to shift the physiological rhythm of phosphate by advancing it by two to three hours.

An earlier phosphate increase could also be due to the parathyrin decrease after calcium administration. Masked by the physiological increase of phosphate, no return to the baseline of phosphate is visible if parathyrin returns to its former concentration.

No significant changes were observed in the calcidiol and calcitriol values. And no statistically significant difference was observed when the concentration of calcitriol (1,25-dihydroxycholecalciferol) before the start of the study was compared with the final evening value (data not shown).

In summary, in our study, all modes of application of a widely used standard calcium preparation lead to a significant influence on the regulation of calcium metabolism. In contrast to i. v. calcium (injection over 5 minutes), the oral administration was without the risk of hypercalcaemia. A dosage increase from 1000 mg to 2000 mg yielded no further increase of serum calcium concentrations. Suppression of intact parathyrin for evaluation of patients with suspected hyperparathyroidism may easily be effected by oral ingestion of 1000 mg calcium. Furthermore, a hyperbolic relationship was observed if total serum calcium (y-axis) was plotted versus intact parathyrin (x-axis), and the effect of serum calcium on parathyrin secretion is much larger if calcium is not elevated, but in the normal range. Single intravenous or oral doses have no significant influence on the vitamin D metabolites, calcidiol and calcitriol.

Oral administration of 1000 or 2000 mg calcium yields an increase in serum calcium concentration, which is within physiological limits, but high enough to significantly influence hormonal regulation. A dosage increase is not accompanied by a proportional increase in the hormonal response.

It has to be stressed that our results refer to a single calcium administration in young healthy adults. The effects of long-term calcium therapy on hormonal regulation, especially in elderly people, still have to be established.

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